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Tintinnids (Ciliophora, Oligotrichea) within power plant discharge and marine protected areas in Masinloc-Oyon Bay

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Abstract

There are only five studies on tintinnids of the Philippines. We present a checklist of tintinnids (Ciliophora, Oligotrichea) from Masinloc-Oyon Bay, Province of Zambales, West Philippine Sea. Masinloc-Oyon Bay is a unique in having both marine protected areas and a coal-fired thermal power plant within the same bay. Field sampling was performed in July 2017, which recorded 10 species belonging to one order, six families, and seven genera. Station 1 inside the power plant's outfall had the lowest diversity, whereas the stations within marine protected areas had a relatively higher species diversity index. Our new data are the first records of tintinnid species in Masinloc-Oyon Bay. These records add to the regional checklist of the Philippine Sea.

Keywords

Bioindicator, marine ciliates, lorica

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Introduction

Microzooplanktonic tintinnids are the most common and widespread group of marine ciliates (Kato and Taniguchi 1993). They are easily identifiable organisms due to their recognizable lorica morphology. They belong to the order Choreotrichida. Tintinnids are also potential bioindicators of water quality and water masses by utilizing their biogeographic classification. These planktonic ciliates have been used by researchers to assess the source of eutrophication and pollution in aquatic environments (Lee and Kim 2010; Kim et al. 2012). Kamiyama and Tsujino (1996) have shown that abundance of tintinnids is correlated to temperature. Pierce and Turner (1993) categorized tintinnids into biogeographic groups namely: cosmopolitan, neritic, warm, tropical Pacific, boreal, and austral species.

Masinloc-Oyon Bay is a livelihood and ecotourism site located in the northwestern Philippines. Studies have been made of its rich marine life, such as corals, fishes, mangroves, seagrasses, and phytoplankton (Furio et al. 2012), but there is no published study of tintinnids in the bay. Masinloc-Oyon Bay has one of the oldest marine protected areas in the Philippines, and it also has a power station (Masinloc Power Partners Co. Ltd.; Masinloc Coal-fired Thermal Power Plant) located on the

northern side of the bay. As tintinnids can be a bioindicator in ecological assessments and as they are easily identifiable organisms compared with other zooplankton groups, tintinnids could be ideal bioindicators for a rapid environmental assessment of a complicated site such as Masinloc-Oyon Bay. Our work is a baseline study of tintinnids in Masinloc-Oyon Bay.

Study Area

Masinloc-Oyon Bay is located on of the island of Luzon about 250 km northwest of Manila (Fig. 1). The 127-ha San Salvador Island Fish Marine Reserve and Sanctuary inside Masiloc-Oyon Bay was established in 1989 under Municipal Ordinance No. 30. The Masinloc-Oyon Bay Protected Landscape and Seascape was proclaimed as a protected area under the Republic Act 11038 or the National Integrated Protected Areas System Act of 1992. The Protected Area Management Board of the Department of Environment and Natural Resources regulates the activities within Masinloc-Oyon Bay. There are four marine protected area (MPA) in Masinloc-Oyon Bay: two MPAs near San Salvador Island where there is a Taklobo (giant clam) farm and a fish sanctuary. The mangrove, *Rhizophora* sp., was planted in what is now the Yaha Mangrove Forest on the south side of Masinloc Bay. Yaha is part of a 115-ha fish sanctuary at Sitio Panglit. Bani fish sanctuary and dive site is another MPA in the northern waters of the bay. The coal-fired power plant in Barangay Bani has operated since its establishment in 1998, and the Masinloc II expansion is now operational.

Methods

Sample collection. Fieldwork was done on 5–6 July 2017 within seven sampling stations in Masinloc-Oyon Bay (Fig. 2). Our collections were made at night from 9 p.m. to 4 a.m. and again in the day from 10 a.m. to 11 a.m. for each station. All sampling was conducted during high tide and at depths of 11–28 m. A zooplankton net (mesh size 20 μm; length 150 cm; mouth diameter 50 cm) was

Figure 1. Map showing the location of the municipality of Masinloc, in Zambales Province, Philippines.

Figure 2. Location of the seven sampling stations within Masinloc-Oyon Bay: (1) near thermal discharge; (2) near power plant but far from thermal discharge; (3) inside Oyon Bay, near fish pens; (4) marine fish sanctuary; (5) giant clam/taklobo farm; (6) near mangrove area; (7) Panglit fish sanctuary.

horizontally towed in surface waters at a depth of 1 m for 10 min with towing speed of 1.5–2.0 knots. Tintinnids from the vertical tow were collected 1 m above the bottom to the surface. A calibrated flow meter was attached to the net to allow estimates of water volume filtered by the net. We collected 50 mL of seawater, which was preserved with 4% formalin. Three sample replicates were acquired at each station. A YSI ProQuatro Multiparameter Meter was used to record water temperature.

Laboratory analysis. The seawater samples were allowed to settle for 48 h in a beaker, and afterwards the upper 25 ml was slowly siphoned off with a dropper. An aliquot of 1 ml from the sample was pipetted into a Sedgewick Rafter chamber. Three aliquots of each sample were prepared, and the mean value was calculated. Tintinnids were identified to the lowest taxonomic level possible under Olympus compound light microscope equipped with Nikon camera (Model: DS-Fi2 K14517). All specimens are stored in the Practical Genomics Laboratory in Science and Technology Research Center, De La Salle University, Manila, Philippines.

For each species, a minimum of five organisms were observed for identification and morphometric measurement. Our identifications were primarily based on lorica morphology, as described by Kofoid and Campbell (1929, 1939), Marshall (1934, 1969), Hada (1937), and Al-Yamani et al. (2011).

Statistical analysis. The following equations were used:

Volume of water filtered. The initial and final reading of the flow meter per tow were recorded. The final reading was subtracted with the initial reading to get the indicated number of revolution. To get the volume water filtered, the indicated number of revolution was multiplied to 0.3m and the net opening area.

$$V_m = \text{indicated number of revolution} \times 0.3\text{ m} \times \text{net opening area in m}^2$$

Subsample count. Every grid of the Sedgewick rafter cell per subsample was analyzed. There are three replicates per horizontal and vertical tow in each station that were counted. The average count of the three replicates was the n = average number of organisms in 1 mL subsample. The V_s or the volume of the plankton sample was 25 mL in all stations and tow. The V_m or the volume of seawater sampled by the net (m^3) was calculated from the equation on the previous section.

$$\text{Subsample} = \frac{n \cdot V_s}{V_m} = \text{no. of organisms/m}^3$$

where n = average number of organisms in 1 mL, subsample V_s = volume of plankton sample in mL, and V_m = volume of seawater sampled by the net in m^3

Shannon species diversity. Species diversity (H′) was calculated by using Pi as the proportion of the total count arising from the ith species, S = total number of species, ln is natural log (Xu et al. 2008).

$$H' = \sum_{i=1}^{S} Pi(\ln Pi)$$

$Pi = ni / N$, where ni is the number of individual of species i and N is the total number of individuals of all species.

Results

A total of 10 tintinnid species were identified and are reported for the first time in Masinloc Oyon-Bay. The two species, *Leprotintinnus simplex* and *Tintinnopsis nana*, are reported from the Philippines for the first time.

Order Choreotrichida
Family Tintinnidae Claparède & Lachmann, 1858
Genus *Amphorides* Strand, 1928

Amphorides quadrilineata (Claparède & Lachmann, 1858)

Figure 3H; Tables 1, 2

Material examined. Philippines • Region III, Zambales, Masiloc-Oyon Bay; 15°33.00′N, 119°55.20′E; 14–15 m; 15°33.50′N, 119°56.13′E; 6–11 m; 15°31.74′N, 119°54.49′E; 25.8–27.6m; 15°29.51′N, 119°56.34′E; 11–12 m; 5.VII.2017; J.A.M Santiago, R.G Santiago leg; MOBAQ1-17.

Identification. Lorica urn-shaped. Anterior part with a round dish-like structure in cross-section. Tall lorica with a concave center that progressively transitions posteriorly into a triangular shape with three conspicuous fins up to the truncated aboral end (Kofoid and Campbell 1929). Greatest width below middle of bowl.

This species differs from *A. minor* Jörgensen, 1924 in being longer and in the structure of the fins (Marshall 1934).

Genus *Eutintinnus* Kofoid & Campbell, 1939

Eutintinnus fraknoii (Daday, 1887)

Figure 3I; Tables 1, 2

Material examined. Philippines • Region III, Zambales, Masiloc-Oyon Bay; 15°33′N, 119°55.2′E; 14–15 m; 15°33.50′N, 119°56.13′E; 6–11 m; 15°31.74′N, 119°54.49′E; 26–28 m; 15°31.94′N, 119°55.50′E; 17 m; 15°29.51′N, 119°56.34′E; 11–12 m; 5.VII.2017; J.A.M Santiago, R.G Santiago leg; MOBEF1-14.

Identification. Lorica relatively large, gradually flaring orally, with conspicuous brim, slightly flaring aborally (Kofoid and Campbell 1929, 1939). Lorica lacking a median bulge (Al-Yamani et al. 2011). Body length 5× oral diameter.

This species differs from *E. lusus-undae* (Entz, 1885) in the presence of an aboral flare.

Eutintinnus lusus-undae (Entz, 1885)

Figure 3J; Tables 1, 2

Material examined. Philippines • Region III, Zambales, Masiloc-Oyon Bay; 15°33.24′N, 119°54.54′E; 13 m; 15°33′N, 119°55.2′E; 14–15 m; 15°31.94′N, 119°55.50′E; 17 m; 15°29.51′N, 119°56.34′E; 11–12 m; 5.VII.2017; J.A.M Santiago, R.G Santiago leg; MOBEL1-21.

Identification. Lorica stout, tubular, almost cylindrical, slightly dilated orally, with low brim. Lorica gradually decreasing in diameter towards aboral end, which shows an inverted and truncated cone (Jörgensen 1924). Aboral flare or brim lacking (Al-Yamani et al. 2011). Body length 3.5× oral diameter. Hyaline wall simple throughout lorica, without any reticulation (Jörgensen 1924).

This species differs from *Eutintinnus fraknoii* in having the lorica shorter, stouter, and without a distinct aboral flare.

Table 1. Morphometrics of tintinnids in Masinloc-Oyon Bay. Bold lettered species are reported for the first time from the Philippines.

Species	Lorica length in μm (n = 5)	Lorica oral diameter in μm (n = 5)
Amphorides quadrilineata	90–160	30–50
Codonellopsis morchella	81–112	30–36
Eutintinnus fraknoii	175–197	34–38
Eutintinnus lusus-undae	140–170	40–45
Favella ehrenbergii	196–239	84–89
Helicostomella longa	40–71	17–21
Leprotintinnus simplex	180–210	30–50
Leprotintinnus nordqvistii	182–243	36–49
Tintinnopsis radix	181–346	35–113
Tintinnopsis nana	30–40	60–70

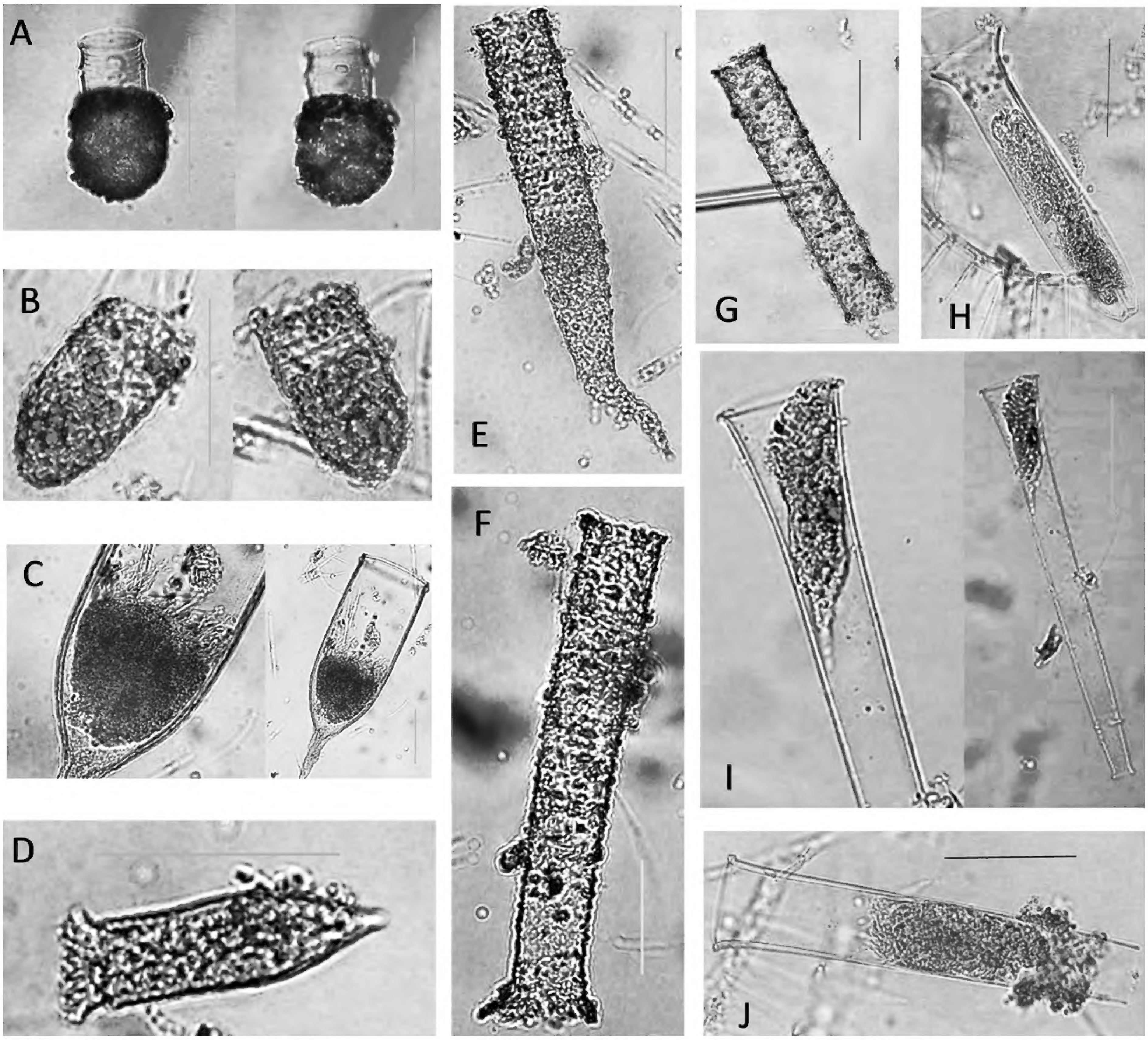

Figure 3. Tinitnnids of Masinloc-Oyon Bay. **A.** *Codonellopsis morchella*; **B.** *Tintinnopsis nana*; **C.** *Favella ehrenbergii*; **D.** *Helicostomella longa*; **E.** *Tintinnopsis radix*; **F.** *Leprotintinnus nordqvistii*; **G.** *Leprotintinnus simplex;* **H.** *Amphorides quadrilineata*; **I.** *Eutintinnus fraknoii:* **J.** *Eutintinnus lusus-undae;* Scale bars = 50 μm.

Family Codonellopsidae Kofoid & Campbell, 1929
Genus *Codonellopsis* Jörgensen, 1924

Codonellopsis morchella (Cleve, 1900) Jörgensen, 1924
Figure 3A; Tables 1, 2

Material examined. Philippines • Region III, Zambales, Masiloc-Oyon Bay; 15°33′N, 119°55.2′E; 14–15 m; 15°31.94′N, 119°55.50′E; 17 m; 15°31.94′N 119°55.50′E; 16 m; 15°29.51′N, 119°56.34′E; 11 m; 5.VII.2017; J.A.M Santiago, R.G Santiago leg; MOBCM1-8

Identification. Collar with flaring rim. Lorica is somewhat bulging near middle, with 8–12 spiral turns and 1–3 elliptical fenestrae. Bowl coarsely agglomerated, oval, with thick wall. Aboral region convex-conical. Aboral end round (Hada 1937; Al-Yamani et al. 2011).

This species differs from *Codonellopsis americana* Kofoid & Campbell, 1929 in having the lorica stouter and bowl ovate.

Family Ptychocylididae Kofoid & Campbell, 1929
Genus *Favella* Jörgensen, 1924

Favella ehrenbergii (Claparède & Lachmann, 1858) Jörgensen, 1924
Figure 3C Tables 1, 2

Material examined. Philippines • Region III, Zambales, Masiloc-Oyon Bay; 15°33′N, 119°55.2′E; 14–15 m; 15°33.50′N, 119°56.13′E; 6–11 m; 15°31.74′N, 119° 54.49′E; 26–28 m; 15°31.94′N, 119°55.50′E; 17 m; 15° 31.94′N 119°55.50′E; 16 m; 15°29.51′N, 119°56.34′E; 11–12 m; 5.VII.2017; J.A.M Santiago, R.G Santiago leg; MOBFV1-22.

Identification. Lorica long, bell-shaped, hyaline (Al-Yamani et al. 2011). Oral rim slightly wavy (Kofoid and Campbell 1929). Collar present on oral portion, with one ring. Upper portion of lorica subcylindrical, posterior part conical. Aboral horn present on posterior end, of varying length and form (Sassi and Melo 1989). Wall

Table 2. Occurrence List of tintinnids in each station in Masinloc-Oyon Bay.

Species	Stations						
	1: near thermal discharge	2: near power plant, far from thermal discharge	3: inside Oyon Bay, near fish pens	4: marine fish sanctuary	5: giant clam/ tak-lobo farm	6: near mangrove area	7: Panglit fish sanctuary
Amphorides quadrilineata	–	+	+	+	–	–	+
Codonellopsis morchella	–	+	–	–	+	+	+
Eutintinnus lusus-undae	+	+	–	–	+	–	+
Eutintinnus fraknoii	–	+	+	+	+	–	+
Favella ehrenbergii	–	+	+	+	+	+	+
Helicostomella longa	+	+	+	+	+	+	+
Leprotintinnus simplex	–	–	+	+	–	–	–
Leprotintinnus nordqvistii	–	–	+	+	–	–	–
Tintinnopsis radix	–	+	+	+	+	+	+
Tintinnopsis nana	–	+	+	+	+	–	+
Total Species	2	8	8	8	7	4	7

bilamellated, with coarse reticulation (Kofoid and Campbell 1929).

This species differs from *Favella campanula* (Schmidt, 1902) Jörgensen, 1924 in have a longer lorica and aboral horn.

Family Metacylididae Kofoid & Campbell, 1929
Genus *Helicostomell*a Jörgensen, 1924

Helicostomella longa **(Brandt, 1906)**
Figure 3D; Tables 1, 2

Material examined. Philippines • Region III, Zambales, Masiloc-Oyon Bay; 15°33.24′N, 119°54.54′E; 13 m; 15°33′N, 119°55.2′E; 14–15 m; 15°33.50′N, 119° 56.13′E; 6–11 m; 15°31.74′N, 119°54.49′E; 26–28 m; 15°31.94′N, 119°55.50′E; 17 m; 15°31.94′N 119°55.50′E; 16 m; 15°29.51′N, 119°56.34′E; 11– 12 m; 5.VII.2017; J.A.M Santiago, R.G Santiago leg; MOBFV1-22.

Identification. Lorica short, bullet-shaped, with oral rim entirely smooth. Suboral turns present. Bowl expanded slightly, with convex conical aboral region. Aboral horn sharply pointed, scarcely differentiated. Body length 3–4× oral diameter.

This species differs from all other species in being smaller and having a stouter lorica (Kofoid and Campbell 1929, 1939).

Family Tintinnidiidae Kofoid & Campbell, 1929
Genus *Leprotintinnus* Jörgensen, 1899

Leprotintinnus simplex **Schmidt, 1902**
Figure 3G; Tables 1, 2

Material examined. Philippines • Region III, Zambales, Masiloc-Oyon Bay; 15°33.50′N, 119°56.13′E; 6–11 m; 15°31.74′N, 119°54.49′E; 26–28 m; 5.VII.2017; J.A.M Santiago, R.G Santiago leg; MOBLS1-6.

Identification. Lorica tubular, slowly tapering to aboral region. Arboral end not flared. Lorica thin-walled, with few foreign particles on surface. Sides more or less curved. Body length 5–6× oral diameter.

This species differs from *Leprotintinnus nordqvistii* (Brandt, 1906) Kofoid & Campbell, 1929 in lacking an aboral flare and from *Leprotintinnus pellucidus* (Cleve, 1899) in bearing no posterior constriction (Kofoid and Campbell 1929).

Leprotintinnus nordqvistii **(Brandt, 1906) Kofoid & Campbell, 1929**
Figure 3F; Tables 1, 2

Material examined. Philippines • Region III, Zambales, Masiloc-Oyon Bay; 15°33.50′N, 119°56.13′E; 6–11 m; 15°31.74′N, 119°54.49′E; 26–28 m; 5.VII.2017; J.A.M Santiago, R.G Santiago leg; MOBLS1-6.

Identification. Lorica tubular, with an inverted funnel-shaped aboral flare. Oral rim with slight flare. Agglomerated lorica decreases gradually in width towards ragged aboral region, flaring outward to aboral opening (Al-Yamani et al. 2011). Body length 4–6× oral diameter.

This species differs from *Leprotintinnus simplex* Schmidt, 1902 in having an aboral flare.

Family Codonellidae Kent, 1881
Genus *Tintinnopsis* Stein, 1867

Tintinnopsis radix **(Imhof, 1886)**
Figure 3E; Tables 1, 2

Material examined. Philippines • Region III, Zambales, Masiloc-Oyon Bay; 15°33′N, 119°55.2′E; 14–15 m; 15°33.50′N, 119°56.13′E; 6–11 m; 15°31.74′N, 119° 54.49′E; 26–298 m; 15°31.94′N, 119°55.50′E; 17 m; 15° 31.94′N 119°55.50′E; 16 m; 15°29.51′N, 119°56.34′E; 11–12 m; 5.VII.2017; J.A.M Santiago, R.G Santiago leg; MOBTR1-25.

Identification. Lorica long, tubular, slender (Al-Yamani et al. 2011). Oral rim ragged, round. Upper region cylindrical and gradually narrowing to aboral region into inverted conical, aboral horn (Jörgensen 1924). Aboral horn slightly curved, with irregular aboral opening (Al-Yamani et al. 2011). Wall thin, with agglomeration of differently shaped and sized particles (Hada 1937). Body length 6–7× oral diameter.

This species differs from *T. cylindrica* Daday, 1887

in having a relatively larger and more slender lorica (Kofoid and Campbell 1929).

Tintinnopsis nana **Lohmann, 1908**
Figure 3B; Tables 1, 2

Material examined. Philippines • Region III, Zambales, Masiloc-Oyon Bay; 15°33′N, 119°55.2′E; 14–15 m; 15°33.50′N, 119°56.13′E; 6–11 m; 15°31.74′N, 119° 54.49′E; 26–28 m; 15°31.94′N, 119°55.50′E; 17 m; 15° 29.51′N, 119°56.34′E; 11–12 m; 5.VII.2017; J.A.M Santiago, R.G Santiago leg; MOBTN1-61.

Identification. Lorica very small, with slightly pointed aboral end. Oral rim ragged. Wall thin, with few agglomerations. Body length 1.5× oral diameter.

This species differs from *Tintinnopsis beroidea* Stein, 1867 in being smaller and in the shape of the aboral portion (pointed in *T. nana*, blunt in *T. beroidea*) (Al-Yamani et al 2011).

Discussion

During our study, sea water temperatures were 30.1–33.0 °C during the day and 29.6–30.7 °C at night. These temperatures are considered warm in the Philippines. At each station, tintinnid species varied in number and makeup. The only species that appeared at all stations is *Helicostomella longa*, which Xu et al. (2012) categorized as a warm-water species. In Liu et al.'s (2010) study in the northern part of the South China Sea, *H. longa* was dominant during summer when temperatures ranged from 20.7–29.6 °C. In our study, the appearance of *H. longa* throughout the bay in July, which is wet but relatively warm, is a valuable finding. This extends the temperature range for *H. longa.*

Higher species diversity (Table 3) was recorded within the two fish sanctuaries and at the giant clam farm. These areas are less disturbed by human activities. These higher tintinnids diversities may indicate that the sanctuaries are promoting resilience within Masinloc-Oyon Bay. The local government unit in Masinloc should continual to preserve these areas. Variability in areas outside of the sanctuaries is higher at night than during the day (Fig. 4) and close to the average for the bay area.

Though our study was limited in duration, the results suggest the need for a more comprehensive survey of the occurrence and abundance of tintinnids and other aquatic organisms in the water column near the power plant. Additional data could help confirm the effects of thermal discharge on the bay. Thermal discharge, along elevated temperatures due to climate change, may negatively affect the marine organisms. There have been accounts showing that powerplant discharges have a negative effect on plankton communities (Chuang et al. 2009; Choi et al 2012), and recent studies have observed that marine plankton may go extinct due to climate change (Trubovitz et al. 2020), and elevated carbon dioxide has multiple impacts on the energy transfer and nutrient and carbon cycling of coastal marine ciliates (Biswas et al. 2012). Additional study of the biological responses of marine organisms in the area of Masinloc-Oyon Bay affected by the powerplant discharge is needed. Environmental advocates have expressed concerns about the power plant's thermal discharge and have called for stricter implementation of environment standards.

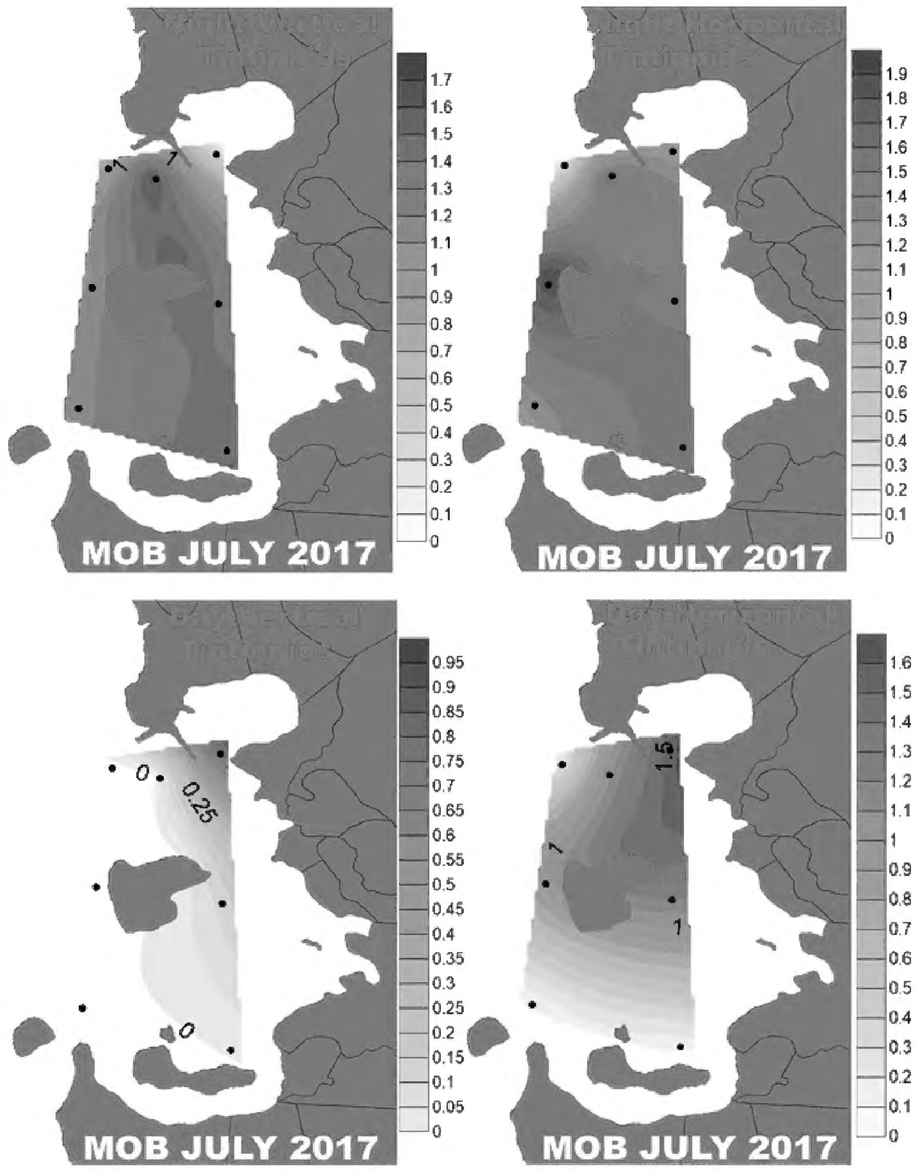

Figure 4. Spatio-temporal distribution of tintinnids diversity index (H′). Dots implicate sampling stations.

Table 3. Summary of the Shannon diversity indices, average abundance, and temperature per collection station of the sampled tintinnids.

Stations	Shannon diversity indices (H′)				Average abundances (ind./m³)	Average temperature (°C)
	Night, vertical	Night, horizontal	Day, vertical	Day, horizontal		
1: near thermal discharge	0.64	0 .00	—	—	13.45	31.67
2: near Power Plant, far from thermal discharge	1.63	1.52	0.03	1.04	18.80	30.21
3: inside Oyon Bay, near fish pens	0 .00	1.05	0.95	1.65	74.36	30.40
4: marine fish sanctuary	1.09	1.90	0 .00	1.07	58.25	30.43
5: giant clam/taklobo farm	1.56	1.33	0.50	1.48	35.11	30.64
6: near mangrove area	1.09	1.09	0 .00	0.05	15.85	30.10
7: Panglist fish sanctuary	1.52	1.61	0 .00	0.08	27.45	30.44

Local government authorities and the power plant's operators need information on the biological effects of temperature within the discharge area and beyond. Additional data would be helpful in determining effects of the thermal discharge and in finding solutions. Transparent water quality assessments at the power plant's outfall should be publicly available. We suggest using tintinnids as possible bioindicators, over time, to monitor unusual, elevated temperatures. As tintinnids are easily identifiable organisms, there is an advantage to using them as bioindicators, in comparison to other zooplankton. Rapid, but reliable, data can still be obtained in a environmental assessment of a complicated marine environment such as Masinloc-Oyon Bay.

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Author's Contribution

Conceptualization: JAS, MCAL. Data curation: JAS. Formal analysis: JAS. Funding acquisition: MCAL. Investigation: JAS, MCAL. Methodology: JAS, MCAL. Project administration: MCAL. Supervision: MCAL. Writing – original draft: JAS. Writing – review and editing: MCAL.

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